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For the Safety of Fresh Produce: Regulatory Considerations for Canada on the Use of Whole Genome Sequencing to Subtype *Salmonella*

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Salmonella is one of the oldest bacteria known to man, yet it is also one of the most prevalent when it comes to foodborne-related diseases and outbreaks. Naturally present in the environment and difficult to treat on fresh produce, *Salmonella* represents an important food safety challenge. Emerging technologies such as whole genome sequencing (WGS) and next generation sequencing (NGS) now offer promising applications within the realm of food safety that can significantly change the way routine testing, inspections and disease surveillance are done. They offer potential avenues that may foster more sustainable agricultural and environmental practices to detect and reduce the presence of *Salmonella*. Strategies are being developed to better cluster, integrate and share genomic data to facilitate the development of diagnostic tests and control methods, as well as generate robust evidence to better inform future policy and regulatory decision-making. Using the approaches developed by the *Salmonella* Syst-OMICS consortium, a large-scale Canadian-based genomic project, this paper discusses the policy and regulatory considerations for the applications of WGS and NGS technologies in the development of testing and biocontrol tools for food safety. The paper presents an overview of the current regulatory framework for the approval of testing methodologies for *Salmonella*. It discusses considerations related to (1) the development of a new test for *Salmonella*, (2) the potential establishment of a *Salmonella* risk virulence classification scheme, and (3) the development of a biocontrol method to reduce the presence of *Salmonella* on fresh produce.

Keywords: biopesticide, food safety, fresh produce, next generation sequencing, *Salmonella*, testing methodology, translational research, whole genome sequencing

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INTRODUCTION

Eating a well-balanced diet integrating fruits and vegetables is known to alleviate the risks of developing several non-communicable diseases including heart-related conditions (Acheson and Williams, 1983; van't Veer et al., 2000; Lock et al., 2005; Wang et al., 2014; Rodriguez-Casado, 2016). In addition to Canadian public health policies and the improvement of agricultural production

practices and global distribution supply chains, there has been an increase in the consumption of fresh and minimally processed ready-to-eat produce in Canada (Allen et al., 2013). However, the consumption of fresh produce has also been correlated with the incidence of foodborne diseases, often resulting in hospitalizations and/or sometimes death (Thomas et al., 2015). Among these foodborne outbreaks in the context of fresh produce, *Salmonella* accounts for 88,000 cases of illnesses per year, the highest incidence of any foodborne pathogen (Ravel et al., 2009; Thomas et al., 2015).

While concerns about *Salmonella* are not new and date back to ancient times (Sánchez-Vargas et al., 2011, p. 264; Callaway, 2017, p. 404; Vågene et al., 2018), the public health approaches to address them over time have led to the sophisticated regulatory systems we know today (Martinez et al., 2007, p. 300). One of the fruits of this evolution is the "farm to plate" framework. This interdisciplinary approach aims to monitor, investigate and control foodborne diseases from the farm (where the food is produced), at the plant (where the food is processed and/or packaged), during its distribution to the final point of sale (nationally and internationally), and at food establishments, where it is served for consumption (Allard, 2002, p. 187; Mahony et al., 2011). A key component of this approach is the technological tools used to support the management of the risks related to pathogen contamination and the enforcement measures that serve to predict, prevent, identify and address potential microbial risk exposure (Lammerding and Fazil, 2000). In this context, the regulatory framework is one of the essential vehicles through which the development and translation of these technologies can best occur.

Using the example of the "Syst-OMICS approach to ensuring food safety and reducing the economic burden of salmonellosis" (hereafter "the Syst-OMICS project"), this paper discusses some regulatory considerations that may arise when technologies such as Whole Genome Sequencing (WGS) and Next Generation Sequencing (NGS) are applied in the context of food safety. More specifically, it discusses how these technologies can potentially be incorporated into current and future efforts to prevent, monitor, control and address *Salmonella* related outbreaks in the context of fresh produce.

THE SYST-OMICS PROJECT

For more than a century, the detection of most bacteria, including *Salmonella*, has primarily relied on culture-based methods, which are held as the gold standard (Köser et al., 2012, p. 2). While these traditional culture-based methods provide a benchmark because they are simple, sensitive and reliable, they also require an important investment in terms of labor and time (Jasson et al., 2010, p. 724). However, current methods of analysis are primarily designed to detect *Salmonella* subspecies and serotypes without differentiating their virulence potential. More recently, additional methods have been developed, including molecular techniques capable of offering more advantages than the sole use of a culture method (Jasson et al., 2010, p. 724). While most of these novel microbiological diagnostic tools have been developed and used in-house rather than for routine testing (Kuhn et al., 2012), further technological breakthroughs are expected to change this, especially with the wider applications of WGS and NGS technologies in the context of food safety. Studies have already shown that these technologies have the potential to generate the data that could serve to better inform decision-making processes related to surveillance and outbreak interventions, research, diagnostics, and the analysis of fresh fruits and vegetables (Little et al., 2012; Ashton et al., 2016; Denis et al., 2016, p. 227). Some of the noted advantages of using these emerging technologies include the improvement of analysis/response time, ease in execution or automation, more precision or accuracy in the results generated, and the reduction of costs (Jasson et al., 2010, p. 724).

In the context of fresh produce, Canadian foodborne outbreaks have mainly been caused by the species *Salmonella enterica* (hereafter *S. enterica*), and its subspecies *S. enterica* subsp *enterica* (Jackson et al., 2013, p. 1239). The study of the diversity of *S. enterica* in humans, animals, foods, and in the environment is essential to understand their evolution, ecology and epidemiology (Little et al., 2012; Emond-Rheault et al., 2017, p. 2). Technologies such as WGS and NGS have the capability to generate the additional data needed to increase our knowledge in this area. For example, they can provide information on the characterization of *S. enterica* genome content, which may contribute to identifying phenotypes specific to strains and serotypes. This, in turn, can help anticipate strain virulence (Yoon et al., 2011), and therefore better inform current practices of diagnostics, epidemiology, and surveillance related to fresh fruits and vegetables (Köser et al., 2012, p. 7; Ashton et al., 2016; Denis et al., 2016, p. 227).

Among the international initiatives to address the challenges presented by foodborne pathogens, the Syst-OMICS project (Emond-Rheault et al., 2017) builds upon the work done by past and ongoing large-scale genomic projects (see for example Freschi et al., 2015; 100k Genome Project, 2017; CDC, 2017; Genomicepidemiology, 2017). The aims of the Syst-OMICS project are to take advantage of the increasing affordability of NGS and WGS technologies to assess *Salmonella* genomes. The project's main research goals include (1) improving and developing testing methodologies to detect *Salmonella* and (2) developing a biocontrol method to prevent or reduce *Salmonella* contamination on fresh produce.

As part of its objective to improve current testing methods, the Syst-OMICS project is investigating whether the virulence potential (i.e., the ability to impair human health and life) of *S. enterica* strains and serotypes can be differentiated between high, medium, low and non-virulent using genomic analysis (hereafter the Syst-OMICS subtyping approach). Thus far, detailed knowledge of the pathogenicity of *S. enterica* serotypes has mostly been derived from a small number of common isolates obtained from laboratory collections (Hoffmann et al., 2014; 2014 p. 1047). Therefore, this project aims to sequence ∼4,500 isolates of *Salmonella* using WGS to examine their genome diversity. The sequences will be annotated with data

on their phenotype, virulence, antibiotic resistance, mobilome gene content[1] and epidemiological data (Emond-Rheault et al., 2017, p. 3). The assessment of the virulence of the strains is particularly important as it may enable a more detailed classification (i.e., high, medium, low and non-virulent). To do so, *Salmonella enterica* virulence categories will be defined by screening isolates for determination of colony forming units (CFUS) and for attachment, adhesion, invasion, and replication in human epithelial and macrophage cell lines. Further screening will be done *in vivo* with infection models using the amoeba *Acanthamoeba rhysodes*, a mouse model indicating animal survival and CFUs in specific organs, and *in vitro* using gastrointestinal fermenter models. The database, SalFos (https://SalFoS.ibis.ulaval.ca/), will serve to improve existing data and potentially inform future diagnostics, epidemiology and outbreak surveillance, and investigation decision-making processes.

Another major objective of the Syst-OMICS project is to develop an ecological biocontrol method capable of reducing or eliminating *Salmonella* on fresh produce. This approach evaluates the potential benefits of using bacteriophages (hereafter the Syst-OMICS biocontrol approach).

While the project has some very promising prospects, the research, design, development and potential adoption of both the Syst-OMICS subtyping and biocontrol approaches are bound to raise considerations that may potentially have broader regulatory implications. To contextualize our discussion, a brief review of the regulatory framework for *Salmonella* is provided before discussing the considerations raised by the Syst-OMICS research trajectories.

THE REGULATION OF TESTING METHODOLOGIES FOR *SALMONELLA* ON FRESH PRODUCE

The Authority to Approve Novel Testing Methods

Food safety regulatory frameworks have evolved over time, with governments progressively integrating standardized, science-based technical methodologies and tools in the development of regulatory instruments (Martinez et al., 2007; Unnevehr and Hoffmann, 2015, p. 2219). In Canada, the *Safe food for Canadians Act* (Canada, 2012) was adopted to further consolidate and coordinate food safety measures across federal agencies and streamline the implementation and sanction of food safety laws, regulations, policies and practices. In the context of microbial testing of fresh produce, two instruments are paramount: the *Food and Drug Act* (Canada, 1985b), which provides for the regulation and management of food standards; and the *Canada Agricultural Products Act* (Canada, 1985a), which provides further directions for the monitoring of fresh produce. The federal government primarily administers these instruments with shared responsibility and jurisdiction with provincial and sometimes municipal authorities (see **Figure 1**).

While not specific to *Salmonella* explicitly, the framework refers to the regulation of microbes and hazardous substances on food and agricultural products. Section 30 of the *Food and Drug Act* (Canada, 1985b), grants federal authority over the inspection of foods, the assessment of the effect of such food on human life and health, and the measures that should be implemented before importing or selling any such food. This is complemented by section 32 of the *Canada Agricultural Products Act* (Canada, 1985a), which specifies federal authority to regulate the analysis, testing and sampling of agricultural products and the regulation or prohibition of the marketing of any fresh or processed fruits or vegetables in import, export or interprovincial trade. The *Fresh Fruit and Vegetable Regulations* (Canada, 2011) further specify that fresh produce is considered contaminated if it contains microbes in contravention to the law or quantities prescribed under the law. Thus, *Salmonella* falls within the scope of these instruments based on the explicit mention and reference in the laws and regulations of microbes and substances that could affect human life or health.

In terms of the testing methodologies for *Salmonella*, while provincial authorities have the ability to develop in-house methods and approve them for regulatory use (see for example section 40 of the *Food Products Act*, Quebec, 2016), the main regulatory approval process is vested in the federal government through Health Canada under the *Food and Drug Regulations* (Canada, 2017). To ensure regulatory compliance, food businesses have also developed their own analytical testing methodologies, notably rapid methods. However, these methods are still required to be at least equal in performance and able to provide equivalent results to methods that have been formally recognized (Jasson et al., 2010, p. 725). Accredited laboratories must use "acceptable" testing methodologies when generating data used for regulatory purposes. Under section A.01.012 of the *Food and Drug Regulations*, the Director (i.e., the Assistant Deputy Minister, Health Products and Food Branch, of the Department of Health) can determine, upon request, whether a method of analysis or examination is acceptable (Canada, 2017, s A.01.012).

The Process for Adopting New Testing Methods

The Microbiological Methods Committee (MMC), which is part of the Bureau of Microbial Hazards (BMH) at Health Canada, has oversight on the approval of microbiological methods used to conduct food safety-related testing, including those used by Health Canada and the Canadian Food Inspection Agency (CFIA). The MMC plays an active role in the harmonization of methods and the coordination of method-related studies, including the collection of data and the conduct of surveys (Health Canada, 2008, p. 9). To review an application, the MMC establishes a technical group (TG) composed of experts from Health Canada and CFIA, who assesses the application

[1]In prokaryotes, "mobilome" refers to all mobile genetic elements that can move between genomes, including transposable elements. This may contribute to the spread of antibiotic resistance and virulence among pathogenic microbes.

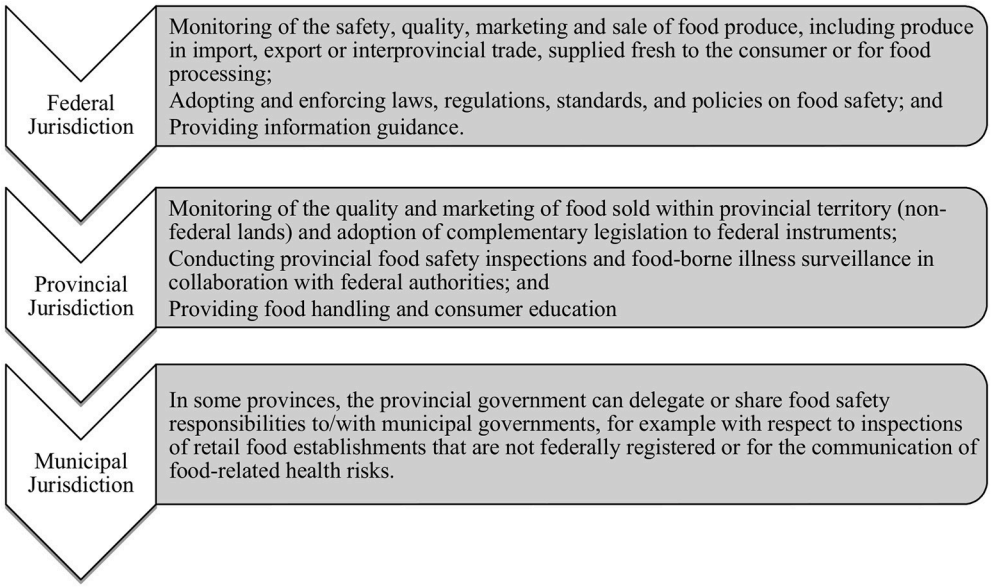

FIGURE 1 | Summary of responsibilities for microbial testing for food safety in Canada.

documents and makes a recommendation to the MMC. This recommendation informs the final decision made by the Director of the BMH (see **Figure 2**).

This process aims to save time, review the method's validation data and verify that the test is fit for the purpose for which the approval is requested. Each method is evaluated on a case-by-case basis and, if needed, additional documents can be provided to supplement the application. These additional documents may include references to Supplementary Materials consulted or used during the development of the method, ISO standards, Health Canada documents, peer-reviewed journal articles, books, other reviews of the methods, and other information on the method including if it has been recognized by another organization in Canada or abroad (Health Canada, 2008). Acceptable methods must meet or exceed specified performance criteria for qualitative or quantitative methods when compared to an accepted method. After their approval and designation as acceptable, these methods are published online in Health Canada's *Compendium of Analytical Methods*.

Methods Currently Approved for the Detection of Salmonella

The *Compendium of Analytical Methods* already includes several methods approved and used by Health Canada, CFIA and other agencies as well as the food industry. Indeed, the testing that CFIA is mandated to conduct (for reporting and investigation) is required to be based on methods that have been approved and included in the *Compendium* and demonstrated to be fit for the related purpose (see for example Denis et al., 2016, p. 227). To date, 12 methods for the microbiological analysis of *Salmonella* have been published in the *Compendium* in *Appendix K* (see **Table 1**).

The regulatory framework remains broad and streamlined enough to foster the development and adoption of new methods, so long as there is robust scientific evidence provided to demonstrate the performance of the method for the purpose sought for approval. Hence, methods improved or developed during the Syst-OMICS project could meet current scientific evidentiary requirements by providing the required supporting science-based data. While generating the required data may be challenging, the approval process of a novel testing method for *Salmonella* does not present any particular regulatory approval issue that would be unique to testing methodologies based on WGS or NGS technologies.

However, the Syst-OMICS subtyping approach, which can be used to develop a new test, presents a novel way of conducting a *Salmonella* risk analysis. This approach could potentially raise some new considerations on how risk analysis for *Salmonella* is integrated within the regulatory framework.

REFINING *SALMONELLA* RISK ANALYSIS APPROACH

In terms of risk assessment, it has been challenging to differentiate between infectious and non-infectious microbial contaminants, especially in the context of fresh produce (Morales-Rayas et al., 2013, 308). Nonetheless, current evidence supports the hypothesis that *Salmonella* serotypes may vary in their virulence. Indeed, only a small proportion of *S. enterica* serotypes is known to be responsible for human infections (Jackson et al., 2013, p. 1239). Among the 46,639 cases of

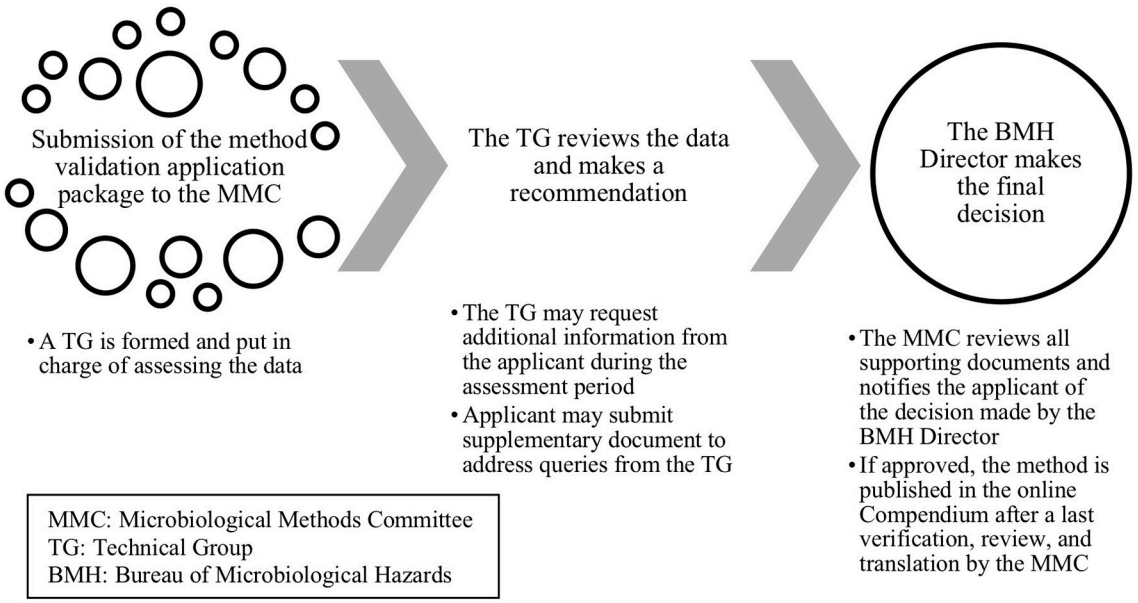

FIGURE 2 | Application process for the approval of a microbiological method for food safety testing.

infection during 1996–2006, Jones et al. identified 687 serotypes, of which 57 were associated with more than 50 cases (Jones et al., 2008, p. 111). Of the most commonly isolated serotypes, 5 accounted together for 61% of all isolates and 13 were more prevalent than *S. Typhimurium* (6%) in cases of invasive diseases (Jones et al., 2008, p. 110). Building upon these findings, the Syst-OMICS subtyping approach proposes to use NGS and WGS technologies to assess whether *S. enterica* serotypes and their subtypes can be identified and classified according to their virulence level as high, medium, and low or non-virulent. As a first step of this analysis, Émond-Rheault et al. have analyzed 3377 *S. enterica* genomes and have been able to distinguish *S. enterica* spp. *enterica* clades A and B phylogeny and a collection of previously defined subspecies (Emond-Rheault et al., 2017, p. 5). The next step is to link phenotypic, epidemiological and clinical *Salmonella* data to the genomic data produced by defining levels of virulence using four different *in vitro* and *in vivo* screening assays as described above.

If research findings were to support the establishment of a virulence classification, this could require policymakers to reconsider what would be the most appropriate regulatory mechanisms for *Salmonella*. As seen in our review of the food safety regulatory framework (see *infra* section 3.1), *Salmonella* is currently regulated from a species classification (as a microbe capable of impairing human health and life). This means that the virulence of specific serotypes and subtypes is not currently used as a significant factor in risk assessment and management approaches for food safety purposes. That is, any ready-to-eat food found to contain *Salmonella* spp. would most likely fall under the classification of a health risk 2 concern and would lead to a food recall. Health risk 2 classification concern situations where a product may cause temporary adverse health consequences or where the probability of serious adverse health consequences is remote; see Canadian Food Inspection Agency, Food Recall and Emergency Responses, Recall Plans—Distributor's Guide, available online at inspection.gc.ca (accessed 18 January 2018). This is understandable given the current lack of information on the actual virulence associated with each known serotype and subtype. Therefore, by filling this data gap on virulence, research findings may support the modification or development of more testing methodologies for *Salmonella*. This in turn may create a need to review and refine current regulatory mechanisms and help identify regulatory measures and interventions that are more appropriate to respond to the risk represented by each potential virulence group (Martinez et al., 2007, p. 303). For example, in the future, we may hopefully be able to recognize some *Salmonella* strains that do not present a threat to human health (because they are of low or non-virulence classification level). The Syst-OMICS preliminary analysis defining levels of virulence as high and low in the four models as well as by genomic prediction of the presence and absence of virulence genes and specific markers supports this hypothesis. Consequently, the results from the Syst-OMICS subtyping approach could potentially inform decision-making processes, policy development and food safety practices related to *Salmonella*.

There is a presumption that with the anticipated data, an appropriate detection mechanism will be in place to integrate the technologies needed to conduct this more precise testing for *Salmonella* virulence. The technological upgrade of testing equipment and methodologies would be a prerequisite to assessing potential regulatory considerations or even any potential regulatory changes. Indeed, institutional environments (at the regulatory agencies, laboratories and within the food

TABLE 1 | Summary of methods approved for *Salmonella**.

Method	Identification number of method	Title
Isolation and identification	MFHPB-20	Isolation and Identification of *Salmonella* from Food and Environmental Samples
	MFLP-75	Procedure for the isolation of *Salmonella* species by the Modified Semi-Solid Rappaport Vassiliadis (MSRV) method
Immunological/Phage technology	MFHPB-24	Detection of *Salmonella* spp. in Foods by the VIDA® SLM™ Method
	MFLP-40	Detection of *Salmonella* in Food Products by the VIDAS® Easy *Salmonella* (SLM) Method
	MFLP-49	Detection of *Salmonella* spp. in food products and environmental surfaces by the VIDAS® UP *Salmonella* (SPT) method
	MFLP-84	Method for the ImmunoMagnetic Separation of *Salmonella* species by Dynabeads™ anti-*Salmonella*
Genetic-based	MFLP-06	Detection of *Salmonella* spp. in Foods using the 3M™ Molecular Detection System Test Kit
	MFLP-20	The GeneQuence® *Salmonella* microwell Assay for the detection of *Salmonella* spp.
	MFLP-29	The DuPont BAX® System Method for the detection of *Salmonella* in foods and environmental surface samples
	MFLP-32	Detection of *Salmonella* spp. using the ADIAFOOD Rapid Pathogen Detection System, A Real-Time PCR Technique
	MFLP-36	Detection of *Salmonella* in Foods and Environmental Surface Samples—Assurances GDS for *Salmonella* Tq Genetic Detection System
	MFLP-38	Detection of *Salmonella* spp. from all foods and selected environmental surfaces using iQ-Check™ *Salmonella* Real-Time PCR Test Kit

**Source: Health Canada, Compendium of Analytical Methods, Appendix K: Summaries of Methods that Detect Specific Bacteria in this Compendium, Salmonella, (Health Canada, 2016).*

industry) and their capabilities to integrate these technological changes have an important part in risk analysis and the efficiency of risk-based regulations and mechanisms (Black and Baldwin, 2010, 194; Unnevehr and Hoffmann, 2015; 2015 p. 2219). Given the decreasing costs of WGS and NGS, it is anticipated that these technologies will be integrated within the food safety systems if they reach cost-efficiency [Thomassin et al., *An Economic Assessment of Salmonella Detection in Vegetables and Poultry: Whole Genome Sequencing Analysis* (unpublished work, 2017)]. However, it is currently difficult to project when such a cost-efficiency threshold will be reached.

Amid this technological integration, the use of a bacterial virulence classification, such as the Syst-OMICS subtyping, could support a more sustainable approach to food safety. Indeed, such a classification could potentially assist with a more rapid, precise and accurate detection of *Salmonella*, especially for those strains which do not pose a health risk. This promising perspective raises a number of questions that could become more pressing once the supportive scientific data becomes available. Among the potential questions, the following two are considered herein: (1) Should fresh produce containing a non-virulent strain of *Salmonella* still be considered "contaminated" for regulatory purposes? and (2) Should fresh produce containing a non-virulent strain of *Salmonella* be allowed for sale?

The first question focuses on how food with non-virulent *Salmonella* should be classified, in light of the premise that non-virulent *Salmonella* would not constitute a threat to human health. Current Canadian legislation mainly focuses on substances and microbes that may be injurious to health. Therefore, non-virulent *Salmonella* may not fall *per se* within the scope of the legislation if the focus is on legislative intent alone, which is to avoid or reduce risk to human health. However, if the focus is on the specific legal terminology used, which refers to microbes (including *Salmonella*), among which some may or may not be virulent, then the regulation would continue to apply and thereby prevent the sale of such contaminated food in the absence of regulatory changes. For example, section 4 of the *Food and Drug Act* states that no person shall sell an article of food that (a) has in or on it any poisonous or harmful substance; (b) is unfit for human consumption; (c) consists in whole or in part of any filthy, putrid, disgusting, rotten, decomposed or diseased animal or vegetable substance; (d) is adulterated; or (e) was manufactured, prepared, preserved, packaged or stored under unsanitary conditions (Canada, 1985b, s 4). Based on the terminology used in the legislation, one could argue that produce contamination attributed to *Salmonella* spp. from an animal source (most likely from the feces of the animal), should be considered unfit for human consumption, as adulterated and/or consisting in whole or in part of a filthy, putrid or disgusting animal substance.

Even if the non-virulent *Salmonella* was to be differentiated from other higher virulence strains, this virulence classification method may not have a significant impact in terms of the regulatory risk classification. In fact, this would not automatically mean that fresh produce contaminated with non-virulent *Salmonella* would be considered differently from produce contaminated with *Salmonella* of a different virulence level or deemed similar to produce that is not contaminated at all.

For example, the presence of non-virulent *Salmonella* may also indicate insufficient processing and thus the potential presence of other pathogens, which may still warrant a food recall. Thus, the use of the Syst-OMICS subtyping could simply imply that "virulence" would be integrated into the regulatory framework as an additional factor in the current risk analysis approach. Food containing non-virulent *Salmonella* could still be classified as "contaminated" for regulatory purposes. This type of approach may be helpful for public health and regulatory long-term tracking and monitoring, as it could provide a transition period to assess or even optimize the use of the virulence subtyping by enabling the collection of additional data.

The second question concerns the potential sale of produce contaminated by non-virulent *Salmonella*. This review of the *Salmonella* risk analysis cannot be done based on scientific data alone. In fact, this assessment needs to be reflective of the scientific advances made, but also of the societal values that serve to contextualize them (Jensen and Sandøe, 2002, p. 253). This discussion of core values is an essential and integral part of the development of regulatory mechanisms and the risk analysis (including risk assessment, management and communication) upon which it is based (Jensen and Sandøe, 2002). Considering that all approaches and methods have both advantages and disadvantages, the objective of this process would be to make optimal use of these scientific findings by assessing how to best exploit the benefits they offer while also taking into account the perception of major stakeholders in the debate (Jasson et al., 2010, p. 728). Finding a balance between the constraints and benefits perceived and conferred may result in different ways of conceptualizing risk levels for stakeholders (Nguyen-the et al., 2016, p. 756).

One such example may be in terms of the financial burden attributed to foods contaminated by *Salmonella*. It has been estimated that salmonellosis costs 1.1 billion dollars a year to Canadians (Todd, 1989, 2014). Amongst the 2,107 foodborne outbreaks that occurred from 1976 to 2005 in Canada, *Salmonella* was the pathogen most frequently associated with the outbreaks (617 outbreaks) (Ravel et al., 2009; p. 1966). More particularly, fresh produce was the food vehicle in 29% of salmonellosis cases (Ravel et al., 2009; p. 1971). A recent study also estimated that *Salmonella* accounts for 50% of bacterial outbreaks associated with produce consumption between 2001 and 2009 (Kozak et al., 2013). In addition, *Salmonella* contamination can also have an impact on the food processing industry and the food service industry (Todd, 1989). In this regard, the use of the virulence classification may have some further implications on the national economy and the international trade of fresh produce, including on both national and international consumers, producers, retailers, chain distributors, and governmental agencies. Given the lack of data on the volume and costs associated with fresh produce discarded or removed from the distribution chain due to *Salmonella* contamination, an investigation should be conducted to estimate such costs, in order to supplement the findings by Todd (1989, 2014) on the national level and those from the FAO at the international level (FAO, 2011). Such studies could provide much needed up-to-date data.

Moreover, an estimation of the proportion of all fresh produce that is likely to be contaminated by non-virulent *Salmonella* should also be conducted. The quantities of produce concerned (small or significant) may inform the need to integrate or not the *Salmonella* virulence classification within the broader *Salmonella* regulatory framework. Even if the amount of food affected was relatively small, due consideration should be given to the advantages it may provide in the context of public health for outbreak surveillance, investigation or intervention. The virulence classification may serve to inform public health officials and regulators about the adequacy of current governance instruments for *Salmonella* contaminated food (i.e., risk assessment, management, and communication).

Should the Syst-OMICS subtyping come to be scientifically validated, it may not be long before similar and/or complementary studies and assessments are conducted in other contexts. For instance, the continued improvement of these testing technologies could serve to improve current practices and potentially help identify and assess other emerging bacterial risks (Jasson et al., 2010). For example, it is well known that there can be large differences in the virulence of different strains of *Listeria monocytogenes* (Hain et al., 2007), and that there are some strains that contain truncated versions of proteins, that essentially make the strain weakly or non-virulent (Jacquet et al., 2004). Hence, these findings could eventually serve to better inform a greater integration of pathogen virulence into future global food safety risk analysis.

The improvement of current testing methodologies and the development of novel methods potentially using a virulence classification system can improve the detection of *Salmonella*. However, once *Salmonella* contamination has been detected, appropriate measures are needed in order to reduce or eliminate the risk to human health.

DEVELOPING AN ECOLOGICAL BIOCONTROL METHOD FOR *SALMONELLA*

The Syst-OMICS project also aims to use WGS to improve the use of bacteriophage-based methods to control *Salmonella* on fresh produce. The particular challenge with fresh produce is that it is often eaten raw or minimally processed, and may not be packaged, which can increase the risk of contamination during the distribution cycle (Hanning et al., 2009, p. 642). The application of strategies that aim at identifying, monitoring and controlling potential points of pathogens entry on fresh produce can contribute to the overall food safety system, but does not necessarily address the issue of what to do with the food that is already contaminated. On this matter, agricultural and environmental concerns were met with little success as decontamination methods on fresh produce have been found to be of limited effectiveness when dealing with *Salmonella* (Hanning et al., 2009, p. 643; Olaimat and Holley, 2012; Tomás-Callejas et al., 2012). Decontamination methods for *Salmonella* contaminated produce such as surface disinfection treatments use chemical compounds (e.g., chlorine water, hydrogen peroxide

treatments), show varying efficiency, may result in the food tasting differently or having altered appearance, and could be harmful to the environment (Olaimat and Holley, 2012; Fong et al., 2017). In summary, agricultural, and public health concerns about these decontamination methods have warranted the exploration of more sustainable and ecological avenues that would be safer to human health and the environment. The use of WGS may also be useful in optimizing different chemical and biological approaches to control the presence of *Salmonella* on fresh produce. These avenues include intervention such as bacteriocins, probiotic cultures and non-thermal inactivation technologies such as high-intensity pulsed UV light. Our discussion however, will focus on one of these approaches: the use of bacteriophages.

Bacteriophages (or phages) are among the living organisms that have the potential to be used as a biocontrol method against bacterial pathogens on contaminated food because of their ability to infect and lyse specific bacteria, and because they are harmless to mammalian cells (USFDA, 2006, p. 5). The use of bacteriophage therapy has been shown to be an effective intervention measure for the control of certain foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria,* and *E. coli* (pathogenic strains) on meat, fresh produce and processed food (Goodridge and Bisha, 2011; Fong et al., 2017). The widespread application of bacteriophages on agricultural products and food intended for human consumption necessitates a selection of phages with suitable characteristics (e.g., virulence, broad host range, reducing phage resistance) (for a more detailed discussion on suitable phage properties, see Goodridge and Bisha, 2011; Mahony et al., 2011; Jassim and Limoges, 2014; Fong et al., 2017).

Nonetheless, there are also some concerns about the use of bacteriophages. Bacteriophage-mediated transduction may serve as an horizontal gene transfer mechanism contributing to the dissemination of antibiotic resistance and virulence genes (Colavecchio et al., 2017). The application of bacteriophages for the control of food contamination may also be limited by a phenomenon known as phage resistance of host pathogens, whereby a bacterial strain may resist infection by a virulent phage (Fey et al., 2010, p. 460). Little is known about the mechanism leading to this phage resistance (see generally Leverentz et al., 2001, p. 1120; Hagens and Loessner, 2007, p. 516; Jassim and Limoges, 2014, p. 2160). However, the use of multi-phage cocktails, compared to a single phage use, tends to diminish the chances of developing such phage resistance (Fey et al., 2010, p. 459; Goodridge and Bisha, 2011, p. 130). Large-scale genomic research such as the Syst-OMICS approach may provide additional knowledge on the phage/bacterial host relation and the characterization and modeling of the emergence of phage resistance. This will contribute to a better understanding of phage-host coevolution and, improve the selection of appropriate characteristics required to use phages as efficient biopesticides (Mahony et al., 2011, p. 161).

When applied as a biocontrol method for food safety and animal health, bacteriophages benefit from less stringent regulation than for human therapeutic applications (see Goodridge and Bisha, 2011; Mahony et al., 2011). A number of bacteriophage-based treatments have been approved by the United States Food and Drug Administration (USFDA) as biopesticides applied to plants or on surrounding soils (Fey et al., 2010). Due to the potential negative implications on human health, the translation from research into routine use and application of phages as a biopesticide in Canada may meet increasing pressure to be included in more comprehensive food safety regulations, which could result in more regulatory requirements (Mahony et al., 2011, p. 158). As such, the regulatory oversight of the use of phages as a biocontrol method would require robust scientific evidence supporting their effectiveness, for them to be integrated into routine agricultural practices, particularly in the context of *Salmonella* on fresh produce.

Hence, the development of biopesticide methods using bacteriophages, such as the Syst-OMICS biocontrol approach, has the potential to help to bridge current information gaps on phages. Thus, robust evidentiary data will be needed to attest to the maturity of these technologies, support their translation into routine use, and strengthen the grounds for their continued environmental and human health applications. Beyond the research and development stage, phage-mediated biocontrol methods have the potential to make a significant impact on preventive and post-contamination treatments of *Salmonella* on fresh produce. At the moment, given the need for additional data, it remains premature to account for the translation of research findings in this area (Fong et al., 2017). Nonetheless, biocontrol methods such as the use of bacteriophage offer opportunities to design agricultural and environmental food safety practices that are more sustainable and ecological. Indeed, these types of methods also present an alternative to the use of synthetic chemicals, which raise a number of concerns, i.e., health concerns related to chemical residues on food, taste, alteration of the appearance of the food, concerns from various stakeholders (industry, farmers, consumers, environmental activists, etc.). Aside from contributing to a more efficient use of bacteriophages as presented in the Syst-OMICS approach, WGS can also be used to monitor the efficiency of other preventive and sanitary control measures and, assist with the optimization of chemicals and biological interventions. For instance, it may help identify which sanitary measures have failed and need to be corrected (USFDA, 2017).

CONCLUSION

Scientific advances are a major source of progress and evolution in today's highly technological society. They have shown us that there is always room for growth, better understanding and opportunities to create and adopt practices that can be more ecological, economical and ultimately more sustainable. In the field of agriculture and food safety, much has been accomplished through the continued improvement of methodological techniques that create predictive modeling of risks related to foodborne pathogens contamination and control. If concerns about *Salmonella* have existed since ancient time,

today, they are accompanied by an increasing demand for rapid pathogen detection methods and a need for alternatives to industrial pesticides, especially in the context of fresh produce. The approaches such as those presented by the Syst-OMICS project build on past studies and have the potential to generate data that will broaden our knowledge in the context of testing and biocontrol tools for *Salmonella*. While such findings may serve to inform future scientific studies and regulatory policies and practices, they also raise a number of questions and regulatory considerations with broad societal implications, including the way *Salmonella* and other pathogens are regulated on produce in the future.

The current regulatory framework is flexible enough to allow for the research and development of improved *Salmonella* detection and control methods. These new tools can receive regulatory approval provided they are mature enough for translation and that robust scientific data demonstrate that they meet regulatory requirements. If research findings support and validate a risk-based virulence classification scheme, this may create a need to review and/or refine current regulatory mechanisms to ensure an adequate regulatory response to address each *Salmonella* virulence category. Alternatively, policy makers may take into consideration a strain's virulence factors in their decision-making processes, for example, as an additional element of a multi-factor approach to *Salmonella* risk assessment and management. Likewise, the use of bacteriophages as a *Salmonella* biocontrol method on fresh produce may provide an environmentally sustainable alternative to chemical approaches. Although agricultural applications of bacteriophages presently benefit from less strict regulation, their approbation for routine use in fresh produce destined for human consumption may require more scientific evidence and regulatory oversight. Such a bacteriophage governance framework could be put in place in Canada by taking into account the experiences of other institutions such as the USFDA (USFDA, 2006; Fey et al., 2010).

Often times, the gap between law and science can be seen as an impediment rather than a vehicle for the efficient translation of sufficiently robust novel technologies from research to routine use. However, ultimately and inevitably, the emergence of novel genomic applications is bound to require greater multidisciplinary collaborations between scientists, lawyers, policy-makers, the food industry and consumers across the translational spectrum for concerted efforts to be fruitful. The development of more efficient detection and biocontrol tools for *Salmonella* offers challenges and opportunities for the provision of additional data, the creation of translational pathways, the review of regulatory requirements, and the assessment of future optimization. Given its long-standing basis in integrating scientific understandings in the assessment of foodborne risks and their management (Unnevehr and Hoffmann, 2015, p. 2219), the food safety framework will be able to integrate the anticipated research findings. This in turn may inform the development or improvement of future regulatory tools. In this light, future studies could contribute to this process through (1) the estimation of the amount of food that may contain only non-virulent *Salmonella* and thereby considered not to present a significant risk to human health; and (2) the estimation of the cost-benefit threshold that could warrant a laboratory equipment upgrade for testing methodologies that would integrate WGS and NGS technologies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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